STUDIES OF THORIUM X APPLIED TO HUMAN SKIN

I. Routes and Degree of Penetration and Sites of Deposition of Thorium X Applied in Selected Vehicles*

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Thorium X is a radioactive substance which has been used in dermatology for almost 40 years. Since the report of Nägeli and Jessner (1) on the dermatologic uses of thorium X, numerous publications have appeared concerning its therapeutic effectiveness and freedom from dangerous sequelae (2). In the past, investigators have been able to study the effects of thorium X on human skin by clinical response and histopathologic changes; only a few speculations have been made concerning the mechanism of its action. A basic problem which has remained unsolved is whether the thorium X is confined to the surface or penetrates the skin in any way. With modern improved technics of studying radioactivity, it is possible to undertake investigations on the routes and degree of penetration and sites of deposition of thorium X. The established usefulness as well as the promise of future usefulness of thorium X in treating numerous dermatoses, skin cancers, and pre-canceroses makes it highly desirable and indeed necessary that information be obtained relative to the mechanism of its biologic effect on human skin.

Thorium X ($_{88}Ra^{224}$),** a natural radioactive material, though a member of the thorium series from which it gets its name, is actually an isotope of radium ($_{88}Ra^{226}$) with which it is chemically identical. The thorium X is procured† as a chloride, adsorbed on $CaCl_2$, in alcoholic (iso-propyl) solution.

The complete radioactive decay scheme is shown in Table I. As the thorium X decays (reaching one-half its original activity in 3.64 days) it emits alpha particles of 5.68 Mev‡ energy. Each atom that disintegrates is transformed to a thoron atom which is also radioactive. Thoron likewise emits alpha particles (energy 6.28 Mev) becoming thorium A.

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A preliminary investigation of this same problem was started several years ago by Dr. Otis Miller (now of Tucson, Arizona) and one of us (VHW). That study was discontinued because the technics available to us at that time were too crude to yield valid results.

We wish to thank Mrs. Eleanor Moreland and Miss Dorothy Carroll for their assistance in this study.

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** Radium, atomic number 88, atomic weight 224.

† From Fleischman, Burd & Co. Inc. 22 West 48th Street, New York 19, N. Y.

‡ Million electron volts.

Thorium A emits alpha particles (6.77 Mev) becoming thorium B, and thorium B emits beta particles (0.36 Mev) becoming thorium C. Thorium C disintegrates to thorium D by one of two alternative routes. Sixty-five percent of the atoms emit first a 2.20 Mev beta particle (plus a gamma) becoming thorium C′ which emits an alpha particle (8.77 Mev). The other thirty-five percent emit first an alpha particle (6.05 Mev) becoming thorium C″ which emits a 1.82 Mev beta particle (plus a gamma). In its complete decay from thorium X to lead (thorium D) a single atom emits a total of four alpha particles, two beta particles and one gamma ray. The chemical properties of the various daughters are those of the elements listed in the second column, titled "symbol".

As a radioactive particle traverses matter it collides with and knocks off electrons from the atoms, of which the matter is composed, leaving a trail of ionized atoms. In each collision the radioactive particle gives up some of its energy. If the path is through a photographic emulsion the resultant ionization causes the silver grains to appear black after routine photographic development and fixation. Thus, if a suitable emulsion* is exposed to a small quantity of thorium X and developed it is possible to see alpha tracks, i.e. the actual paths traversed by the alpha particles in the emulsion.

TABLE I

Thorium X Disintegration Series

NAME	SYMBOL	HALF-LIFE	ENERGY OF RADIATION IN MEV		
			Alpha	Beta	Gamma
Thorium X	$_{88}Ra^{224}$	3.64 days	5.68	—	—
Thoron	$_{86}Rn^{220}$	54.5 sec.	6.28	—	—
Thorium A	$_{84}Po^{216}$	.158 sec.	6.77	—	—
Thorium B	$_{82}Pb^{212}$	10.6 hrs.	—	0.36	—
Thorium C	$_{83}Bi^{212}$	60.5 min.	6.05	2.20	?
65% → Thorium C′	$_{84}Po^{212}$	3×10^{-7} sec.	8.77	—	—
35% → Thorium C″	$_{81}Tl^{208}$	3.1 min.	—	1.82	2.62
Thorium D	$_{82}Pb^{208}$	Stable	—	—	—

The number of collisons per unit length of path depends upon the density of material (number of atoms per unit volume) traversed and the mass of the radioactive particles. In a dense material such as a photographic emulsion an alpha particle undergoes frequent collisions, rapidly losing its energy and coming to a stop. The most energetic alpha particle in the thorium X chain (8.77 Mev) has a range of about 8.6 cm. in air and only 50 microns in a photographic emulsion. The much lighter (1/7000) beta particle causes less ionization per unit path and thus travels about 100 times farther.

There is a separate and distinct track formed by each alpha particle. Since the density of ionization due to beta particles and gamma rays is much less than that of alpha particles, their tracks do not show up in our autograms. However special technics can be applied to show their effects when this is desired.

The four alpha particles given off by a thorium X atom and its daughters may be emitted at random in any direction. On the basis of statistical probability, one-half of the total number of alpha particles emitted will travel away from the emulsion and one-half will travel into it. If the parent atom is in the emulsion or at the surface and all four rays travel into or through the emulsion and are recorded photographically, a characteristic four pronged star will appear, the center of which corresponds to the nucleus from which these alpha particles came. Thus a four pronged star must represent a thorium X atom

* We used Kodak NTA emulsion, 25 microns thick.

which has decayed, with emission of four alpha rays, to lead. However single tracks or two or three pronged stars may actually represent thorium X atoms from which some alpha particles have traveled away from the emulsion and therefore were not photographically recorded. If the disintegrating atom is located in the tissue above the tissue-emulsion interface the center of the star is not *in* the emulsion and only single tracks appear.

If one starts with thoron, the maximum number of branches in a star is three, as there are only three alpha particles emitted in the decay of thoron to lead. Thus a three pronged star must be considered to originate from thorium X or thoron. By similar reasoning, two prongs can originate from thorium X, thoron or thorium A, and single tracks from thorium X, thoron, thorium A or thorium C or C′.

PROCEDURE

Human skin for this study was obtained from patients who were having biopsies performed for diagnostic purposes at the Skin and Cancer Unit of New York University Hospital. When elliptical scalpel excisions were to be done, a portion of normal skin adjacent to the diseased tissue was utilized for our studies. The part to be excised and the surrounding area was cleansed well with 70% alcohol. A small (about 5 mm^2), roughly triangular, area next to the pathologic tissue was walled off with rubber or Duco cement. Thorium X in alcoholic solution was applied to the walled-in area with either a micropipette or a cotton tipped applicator, *without rubbing.* The amount applied varied from 1 to 15 lambda* and the strength from .06 to 3.0 microcuries. The entire area to be excised was then covered with collodion and allowed to dry, after which a dry dressing was applied as an additional protective covering. The patient was instructed not to wet the area. One to five days later the collodion was stripped off, taking with it the rubber or Duco cement. A faint, fairly well defined erythema usually marked the treated area. The region was then anesthetized with 2% solution of procaine using peripheral infiltration block in most cases, although injection beneath the area was employed in some few instances. To avoid spreading the thorium X the area was not cleansed with alcohol or other antiseptics prior to excision. During the procedure, however, it was not always possible to avoid the free flow of blood across the surface of the skin and the area treated with thorium X. The excised tissue was gently blotted dry between pieces of gauze, and taken to the laboratory for preparation and sectioning.

Technics generally employed, such as fixing in Bouin's solution or formalin, or dehydrating in alcohols in preparation for embedding in paraffin, were discarded because of leaching out or displacement of thorium X and its daughters. Instead, tissue was either fixed in a paraformaldehyde gas chamber for 3–6 hours according to the technic of MacKee, Herrmann, Baer and Sulzberger (3), or the unfixed tissue was frozen directly, before sectioning on the freezing microtome.

In the early phases of the work the cut sections were floated on to the photographic emulsions with water. Autoradiograms prepared by this method were useable, but there was in most instances a confusing amount of scattered activity which was thought to be due to the water flotation. Alcohol, acetone, xylol and propylene glycol flotation were tried and likewise discarded in turn.

* One lambda is equivalent to one-thousandth of a cubic centimeter.

The most satisfactory method tried thus far has been that of Adamstone and Taylor (4)*. The freshly excised tissue was mounted without the use of water on the object disc of a freezing microtome. The sections cut from the frozen tissue with a blade kept cold by solid CO_2 were transported to the photographic emulsion in a frozen state on a specially constructed shovel packed with solid CO_2. The moment the tissue came in contact with the warm emulsion it thawed and adhered to the gelatin. This was the only step of the procedure at which moisture appeared on the tissue.

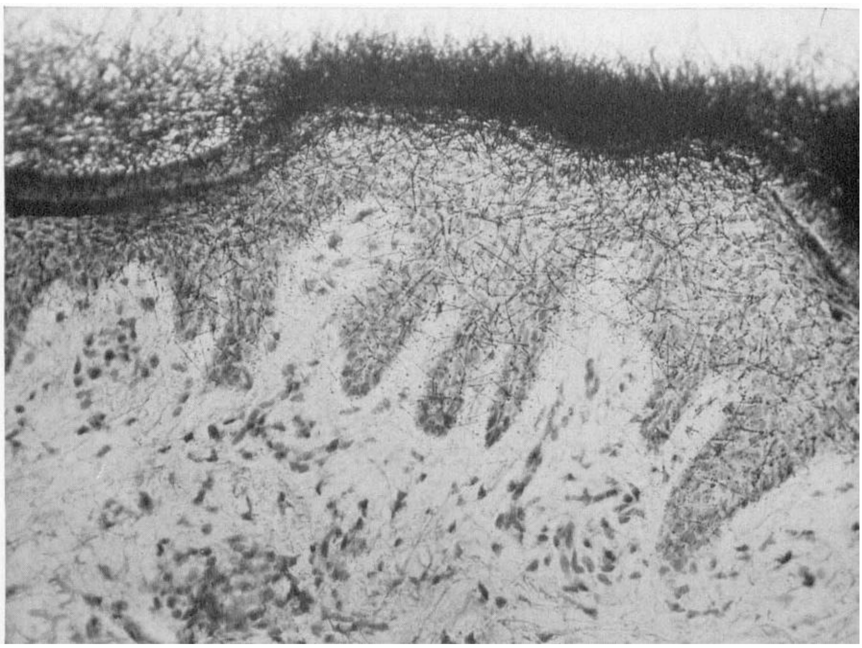

FIG. 1. A section of human skin biopsied five days after topical application of alcoholic solution of thorium X. Cut perpendicular to the epidermal surface. The activity is particularly dense on the surface but is also clearly noted through the prickle and basal cell layers. There are essentially no alpha tracks in the dermis. (Magnification: 330×).

Note: Histologic detail is lacking because the critical focus in this and all of the photographs which follow is on the tracks in the 25 micron emulsion *beneath* the level of the tissue section.

Tissue to be cut perpendicular to the surface of the epidermis was mounted on the object disc of the freezing microtome with the long axis of the epidermis at right angles to the knife blade. For sections parallel to the surface of the epidermis, the tissue specimen was cut starting in the cutis and working toward the stratum corneum. Practically all sections were cut 15 microns thick.

The photographic plates with the adhering tissue sections were exposed from

* We are indebted to Dr. Herbert Mescon for his kindness in demonstrating this technic to us.

4 hours to several days. They were then developed in Kodak D-19 developer for 4 minutes, washed in running water for 10 minutes, and fixed in Kodak F-5 hypo for 45–60 minutes. The plates were again washed in running water for 1 hour and dried in a dust proof cabinet containing Drierite.

A modification of the method of Simmel, Fitzgerald and Godwin (5) was employed for staining. The dried slides were immersed in a solution of metanil yellow for 10 seconds, washed in a mixture of equal parts of absolute alcohol and xylol, dried rapidly by blowing, immersed in the specially prepared solution of iron hematoxylin for 3–5 minutes, washed again in the alcohol-xylol mixture and

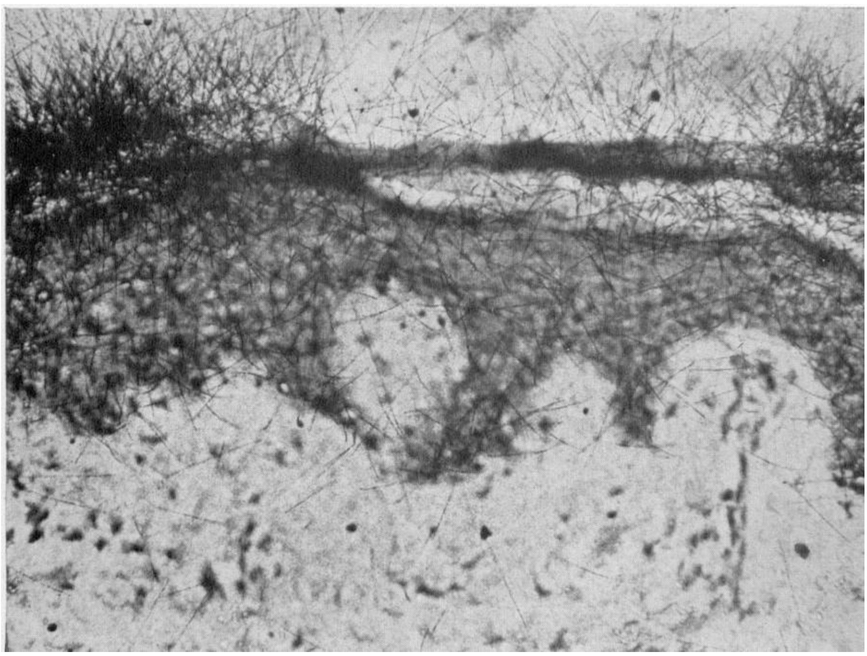

FIG. 2. A section of human skin biopsied 24 hours after topical application of alcoholic solution of thorium X. Cut perpendicular to the epidermal surface. Note that alpha tracks are most dense at the epidermal surface, but also appear in the epidermis down to the basal cell layer, including the rete pegs. The sparcity of tracks in the cutis is quite apparent. (Magnification: 500×).

again dried rapidly by blowing. The stained sections and the emulsion were then protected by a glass cover slip held in place with gum damar.

RESULTS

Autoradiograms were prepared from sections of nine pieces of tissue taken from eight different patients. The photomicrographs shown in Figures 1 through 8 are representative examples of the results which were obtained. Histologic details are lacking in all of the photomicrographs, as the critical focus is on the tracks *at one plane* in the 25 micron-thick emulsion, beneath the level of the tissue section. For the same reason those tracks which travel obliquely through the emulsion are therefore out of focus and appear blurred.

All freshly biopsied pieces of skin with the exception of specimen 2 (Figure 2) were immediately sectioned on the freezing microtome. The frozen sections were transferred directly to NTA emulsion. Specimen 2 was fixed in a paraformaldehyde gas chamber for two hours before it was frozen. Sections cut from this specimen were floated on water to NTA plates.

The autoradiograms prepared from the sections of skin which had been previously treated with thorium X in alcoholic solution show the greatest accumula-

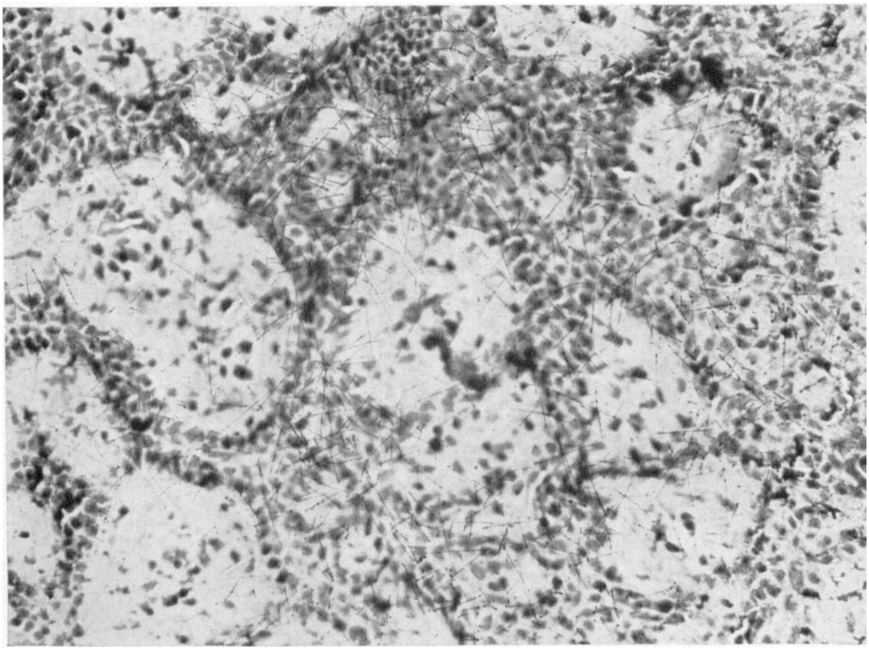

FIG. 3. A section of human skin biopsied five days after topical application of alcoholic solution of thorium X. Cut parallel to the epidermal surface. The epidermal rete pegs show numerous alpha tracks. The dermis of the intervening papillae shows only occasional tracks. (Magnification: 360×).

tion of activity to be at the surface (Figures 1 and 2). The activity in the remainder of the epidermis is rather evenly distributed through the prickle and basal cell layers. This is illustrated in Figures 1, 2 and 3. In specimens 1 and 2, which were cut perpendicular to the epidermal surface, alpha activity is seen to extend from the superficial layers of the epidermis through the length of the rete pegs. In Figure 3 the specimen is cut parallel to the surface and again it is quite evident that the activity is limited essentially to the epidermal tissues (rete pegs).

In the preparation of these parallel sections the microtome knife blade did not cut through the surface of the epidermis or any other large area where there might be deposition of a large amount of radioactivity.

The accumulation of alpha activity in deeper epidermal structures is shown in Figures 4 and 5. In these sections of hair follicles cut parallel to the epidermal surface, the greatest activity is seen in the central follicular space (particularly Figure 4), but also is localized in the epidermal tissue making up the follicular appendage.

Even deeper penetration of epidermal structures by thorium X in alcoholic solution is shown in Figures 6, 7 and 8. Here alpha activity is seen confined essentially to sweat ducts as deep as 1.4 mm. below the epidermal surface. Alpha

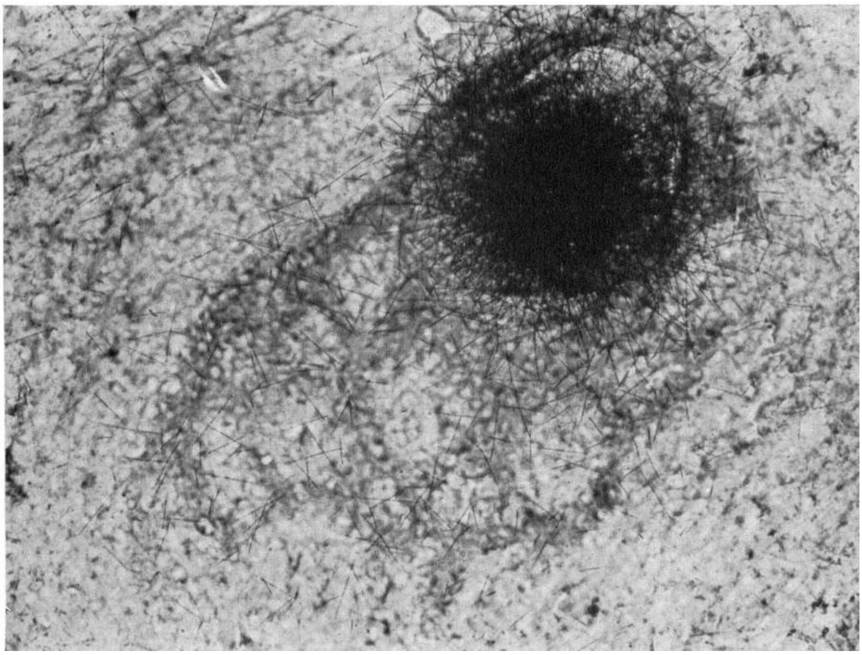

FIG. 4. A section of human skin biopsied two days after topical application of alcoholic solution of thorium X. Cut parallel to the epidermal surface. A dense accumulation of activity is seen within the central portion of a hair follicle. There is an apparent localization of alpha activity in the epidermal tissue adjacent to the hair follicle. In addition, there are scattered tracks in the cutis. (Magnification: 360×).

activity in a group of sweat glands is shown at 1.8 mm. below the surface of the epidermis (Figure 8).

In all of the photomicrographs some alpha tracks are seen in the dermal tissue. These tracks are most numerous adjacent to the basal layer of the epidermis and surrounding epidermal structures (hair follicles, sweat ducts and sweat glands). Several possible explanations for this finding are given in the discussion which follows.

The localization of alpha activity as demonstrated in the above photomicrographs was reproduced many times in autoradiograms made from serial sections. All nine pieces of tissue showed similar localizations of radioactivity, although some specimens showed a particular localization better than did others.

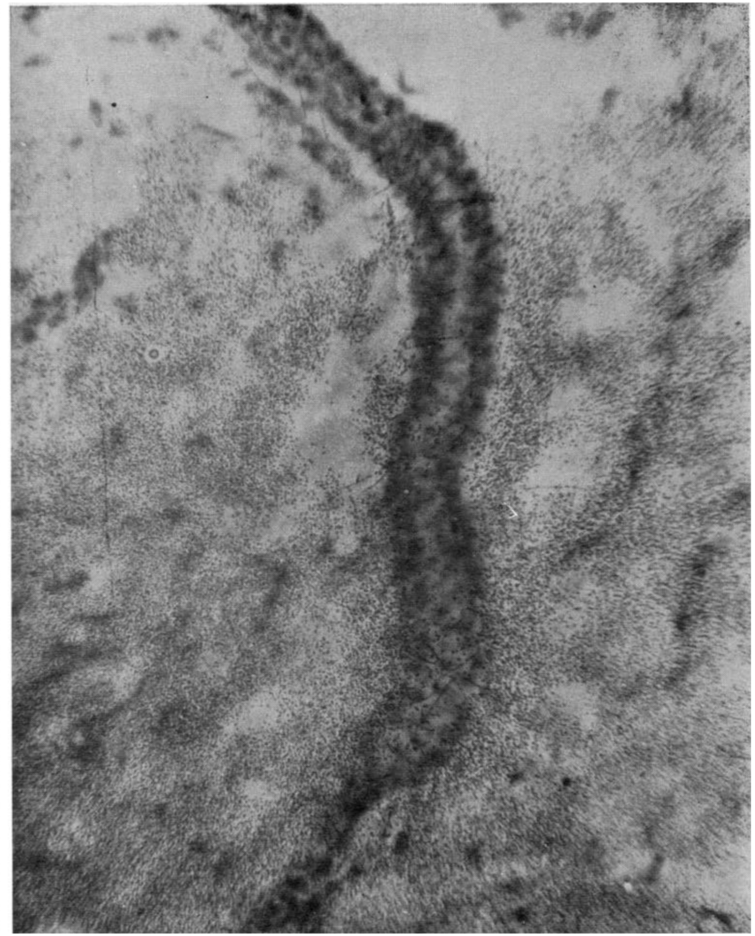

FIG. 5. (Left) A section of human skin biopsied 48 hours after topical application of alcoholic solution of thorium X. Cut parallel (oblique) to the epidermal surface. Alpha tracks are fairly well confined to the epidermal structures of the hair follicle and within the follicular space. (Magnification: 360×).

FIG. 6. (Right) A section of human skin biopsied 2 days after topical application of alcoholic solution of thorium X. Cut perpendicular to the epidermal surface. Alpha tracks are noted along the entire course of the sweat duct (measured to a depth of 1.4 mm. below the epidermal surface) and are shown confined almost entirely to this structure. The bubbles are an artifact. (Magnification: 500×).

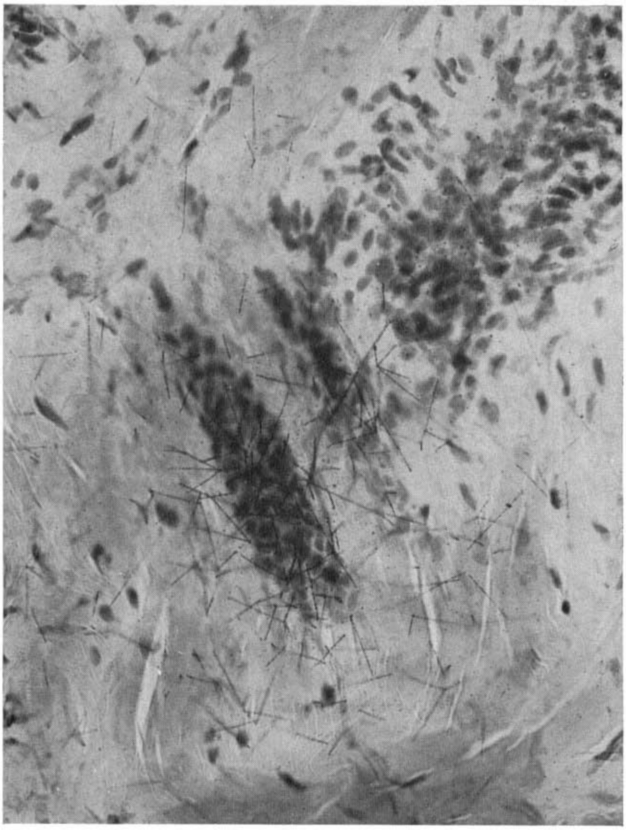

FIG. 7. A section of human skin biopsied 2 days after topical application of alcoholic solution of thorium X. Cut perpendicular to the epidermal surface. Alpha activity is seen localized in and around a sweat duct 0.85 mm. below the epidermal surface. This localization was reproducible on serial sections. Note the numerous multiple pronged stars. (Magnification: 500×).

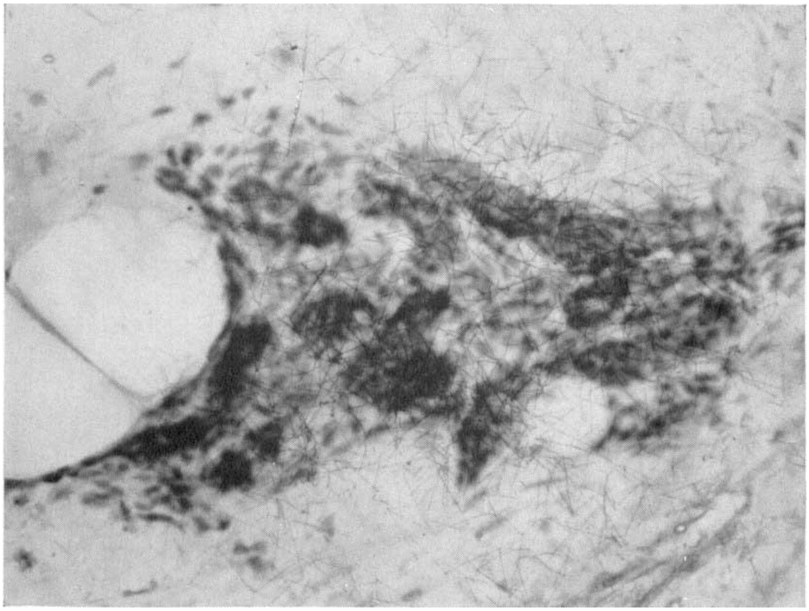

FIG. 8. A section of human skin biopsied five days after topical application of alcoholic solution of thorium X. Cut perpendicular to the epidermal surface. Activity is seen within and surrounding the group of sweat glands which are 1.8 mm below the epidermal surface. (Magnification: 440×).

In addition to single tracks, two-, three- and four-pronged tracks are also found in the epidermal and dermal tissue. Whereas these are best seen through the microscope where the three dimensional aspects can be studied, they are also visible in the photographs.

DISCUSSION

Lomholt (6) in 1923 speculated on the possible causes of the histopathologic changes which he noted as deep as 500–600 microns from the surface in human skin treated with thorium X. Assuming that the effect was due to the alpha radiation and that the alpha rays could not penetrate more than 100 microns in tissue, he considered the possibilities of 1) the penetration of the thorium X itself, 2) the penetration by diffusion of the thoron gas or 3) the effect being "of some other kind than their ionizing power". From his own studies he eliminated the first and last of these, concluding that the diffusion of the thoron gas into the depths of the skin was responsible for the biologic effects noted.

Although we have not attempted to study or explain the mechanism of the action of thorium X on human skin, our results do indicate that when thorium X is applied *in alcoholic solution*, some of the parent substance does penetrate the epidermis into the basal layer including the rete pegs, passes down the hair follicles, and is found in the sweat ducts and sweat glands.

Lomholt's experiments (which utilized thorium X chloride dried on a thin metal foil and applied to the skin to produce erythema) were interpreted by him to mean that it was the thoron gas which was doing the penetrating. Whereas the gaseous emanation may diffuse through the tissue, we found thorium X, as evidenced by four pronged stars, in all places where we found concentrations of alpha tracks. This suggests the possibility that even in Lomholt's experiments the soluble thorium X chloride, when in close contact with the skin, may have been taken into solution by the perspiration and other solvents collecting under the foil and then carried down into the tissue in a fashion similar to that demonstrated in our studies with thorium X in alcoholic solution.

Whereas some alpha activity is found in the cutis adjacent to the basal layer and surrounding the hair follicles, in the sweat ducts and the sweat glands, we are unable, as yet, to state whether this activity is due to an *in vivo* penetration of the structure by the vehicle bearing the thorium X or whether it is due to *in vitro* displacement caused by technic.* If the former is correct, it might be explained by the mechanisms of percutaneous penetration as demonstrated by MacKee, Sulzberger, Herrmann, and Baer (7), i.e. the penetration of the hair follicles and sweat ducts and glands by the solution carrying the thorium X. Our results also suggest that there may be a direct penetration of the full thickness of the epidermis by this radioactive substance in alcoholic solution.

* The presence of multiple star centers within the emulsion indicates conclusively that there is some *vertical* displacement of thorium X from the tissue into the emulsion. It is therefore reasonable that *lateral* displacement may also occur during the autoradiographic procedure. The presence of tracks in the emulsion surrounding epidermal structures is probably the result of such lateral displacement from these structures.

Our findings, which demonstrate the localization of alpha activity in the epidermis, hair follicles, sweat ducts and sweat glands on single autograms, are repeated many times on serial sections done both perpendicular and parallel to the epidermal surface.

The appearance of track concentrations in corresponding areas of serial sections confirms our conclusion that the localization described is real and not due to artifacts. However it is undeniable that artifacts do occur; and in the beginning they made proper interpretation of our autoradiograms difficult.

Among the many possible causes of such artifacts the following are of particular importance: 1) the folding of tissue sections, 2) tearing off and displacement of all or part of the superficial layers of epidermis, 3) displacement of cells, sebum and/or debris, etc. containing radioactive material from any part of the tissue to another, 4) radioactive atoms being carried from one site to another by the microtome blade, and 5) unavoidable contamination. With refinement of technics and additional studies, it should be possible to improve and extend our findings and interpretations.

SUMMARY

1. Introductory remarks are made concerning some of the properties of thorium X.

2. The procedures used in preparing the autoradiograms from human skin treated *in vivo* with thorium X in alcoholic solution are described in detail. Such studies were done on nine pieces of tissue taken from eight different patients.

3. Using the described autoradiographic technic our studies demonstrate for the first time that thorium X *in alcohol solution:* 1) Penetrates the epidermis and is distributed through the prickle cell and basal layers; 2) Enters the hair follicle and penetrates the follicular wall; 3) Enters the sweat duct and is found within the sweat gland.

Whereas some alpha activity is found in the cutis, we are unable, as yet, to state whether this activity is due to *in vivo* penetration or *in vitro* displacement.

4. The findings are discussed and some of the possible sources of artifacts are described.

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DISCUSSION

DR. MARION B. SULZBERGER: The presentation by Dr. Witten and his co-workers could scarcely do justice to the tremendous amount of work and patience and skill which were needed to achieve these results and to produce the beautiful autoradiographic pictures. The present findings are actually the outcome of a very long series of attempts with a great diversity of methodological variations and are merely preliminary to other studies.

I who have been privileged to watch over this work from its inception want to express my personal admiration for the way in which Dr. Witten and Miss Oshry and their efficient collaborators, Dr. Ross, Dr. Hyman and Miss Holmstrom, have attacked and solved the many technical problems and succeeded in eliminating many sources of error. They have displayed great ingenuity in developing new technics which permit the autoradiographic demonstration of where the applied thorium X and the alpha particles actually go in the cutaneous tissues; and which rule out many artifacts which, with older technics, would lead to erroneous conclusions due to artificial displacement of the applied materials. It is for this reason that the present studies are to be regarded not only as applicable to the problem of Thorium X but as preliminary to many other fundamental studies with autoradiographic technics in dermatology. Dr. Witten and his team have achieved the development of more accurate tracer methods which can, and undoubtedly will, be applied to a great variety of fundamental dermatologic problems and to autoradiographic studies in general.

DR. WITTEN: I wish to thank Dr. Sulzberger for his kind remarks. May I also thank Miss Holmstrom and Miss Oshry for their assistance in the presentation and express my appreciation to the Technicon representatives for making the projection of the slides possible.